

Effects of denervation on palmar sweating in the Rhesus monkey (*Macaca mulatta*)

By A. A. SMITH

School of Podiatric Medicine, Barry University, Miami Shores, Florida, USA

*Receipt of Ms. 16. 6. 1992
Acceptance of Ms. 29. 4. 1993*

Abstract

Observed the effects of denervation on the response of eccrine sweat glands to exogenous methacholine. Interprets these results in the light of recent studies of sweating.

Cutting the nerve supply to the eccrine sweat glands of 3 female rhesus monkeys ended spontaneous production of visible sweat. Within 2 weeks the sweat glands' capacity to produce visible sweat in response to sudorific drugs is also lost. The nerves visibly degenerate over a 1 month period. These effects are like the effects of cutting the nerve supply to human eccrine sweat glands.

The denervated sweat glands remained histologically normal throughout the 5 weeks of observation. They also continued to show phosphorylase a activity, which is normally associated with sweat production. This suggests that denervated sweat glands produce water vapor. Other studies of monkey and human sweat glands provide further evidence of vapor sweating in both denervated and normal sweat glands.

Vapor sweating is an efficient cooling mechanism for a furry animal. The spread of eccrine sweat glands from the palms and soles to the general body surface over the course of the evolution of higher primates was due to the survival value of vapor sweating.

Introduction

Most mammals have eccrine sweat glands, which secrete clear, odorless sweat, only on the palms and soles (KUNO 1956; MONTAGNA and PARAKKAL 1959). Like man, the apes and monkeys of the Old World (MONTAGNA and YUN 1962; MONTAGNA et al. 1964; MACHIDA et al. 1964) and the higher New World monkeys (PERKINS and MACHIDA 1967; MACHIDA and GIACOMETTI 1968) also have eccrine sweat glands on the general body surface.

Eccrine sweat glands are normally under the control of cholinergic nerves (DALE and FELDBERG 1934), although they also have some adrenergic innervation (UNO and MONTAGNA 1975).

The eccrine sweat glands on the soles of cats (NAKAMURA and HATANAKA 1958) and lemurs (SAKURAI and MONTAGNA 1965) still respond to injected acetylcholine after the nerve supply is severed. Human eccrine sweat glands, even those on the palms and soles, require an intact nerve supply to produce visible sweat in response to sudorific drugs (COON and ROTHMAN 1941; SILVER et al. 1963). The sweat glands of the African green monkey, *Cercopithecus aethiops*, also become unresponsive after denervation (SAKURAI and MONTAGNA 1964).

This study was undertaken in hope that histochemical observations on the glands after denervation would help to elucidate the mechanism of the loss of responsiveness. Several recent studies show that this data also suggests how thermoregulatory sweat glands could have evolved in furry animals.

Material and methods

Three female rhesus monkeys, *Macaca mulatta*, were used in this study. The median, ulnar, and radial nerves in one upper arm of each monkey were severed (and sutured to allow regeneration after the experiment) under phencyclidine anesthesia.

The palm and fingertips of the operated hands were tested for spontaneous sweating before and after the operation by pressing bromphenol blue paper (SAKURAI and MONTAGNA 1964) against them. At the same time a different fingertip or palmar pad on the denervated hand of each monkey was injected with 20 μ g acetyl- β -methylcholine chloride in 50 μ l saline to test the sweat glands' capacity to sweat. Liquid sweat was visualized by pressing bromphenol blue paper against the injected area. Similar observations were made on the unoperated palms.

Biopsies were taken (under phencyclidine anesthesia) from the denervated palms immediately before the nerves were cut and at irregular intervals thereafter. A quarter of each biopsy was fixed in Helly's fluid, dehydrated in Lugol's iodine, embedded in Paraplast, sectioned at 7 μ m, and stained with the PAS technique followed by hematoxylin (LILLIE 1954).

A second quarter of each biopsy was frozen in liquid nitrogen, sectioned in a cryostat at 20 μ m, and incubated in the medium of ERÄNKÖ and PALKAMA (1961) without adenylic acid to show phosphorylase a activity. Staining was preserved by mounting in iodized "Histoclad" (SMITH et al. 1966).

Frozen sections were also incubated in the medium of BURSTONE (1961) to show cytochrome oxidase activity. The rest of the biopsy was fixed for 1½ hours in 10% formalin at 4°C, sectioned at 40 μ m, and incubated 5 hours in a solution of cupric sulfate and acetylthiocholine iodide to show cholinesterase activity (GOMORI 1952). Some of these sections were counterstained with hematoxylin.

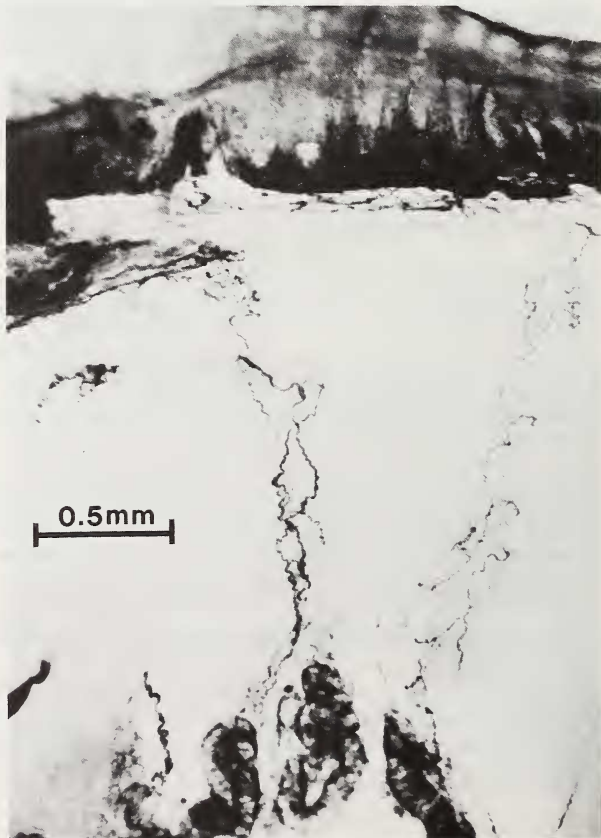


Fig. 1. Cholinergic nerves around sweat glands and their connections with the subpapillary plexus. Acetylthiocholine iodide - CuSO_4 . $\times 40$

Surgery and post-operative care were in conformity with the U.S. Animal Welfare Act of 1966. For a few months after the surgery, the animals required care like that given to a human with similar nerve lesions due to a misused tourniquet. Although such experiments were common at the time of this work, I would not repeat this experiment today.

Results

The palms of two monkeys were sweating spontaneously before the operation. None of the operated palms sweated spontaneously immediately after denervation. Spontaneous sweating reappeared in two of the operated palms during the first week after denervation. Spontaneous sweating was never seen in a denervated palm after the end of the first week.

All 3 unoperated (control) palms sweated spontaneously after the operation.

All of the denervated palms continued to sweat in response to acetyl- β -methylcholine for the first week after denervation. During the second week all of the denervated palms ceased to respond. The control palms always responded.

Cholinesterase-positive nerve fibers surrounded the secretory coils of the eccrine sweat glands. Almost all of the fibers were circular rather than longitudinal and lay in a single surface of revolution just outside the fibroelastic capsule of the gland. A few unoriented

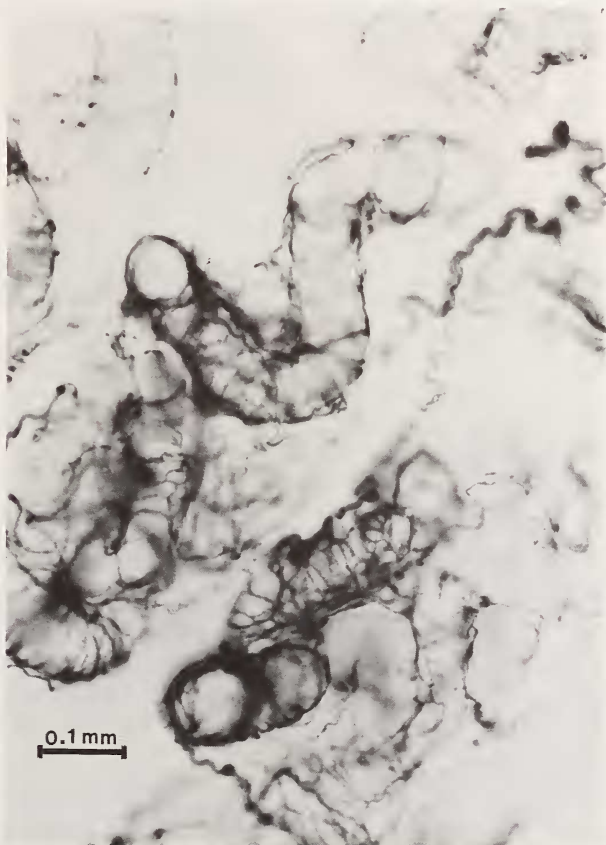


Fig. 2. Three days after denervation the cholinergic nerves around the secretory coil show no visible change. Acetylthiocholine iodide - CuSO_4 , $\times 130$



Fig. 3. Two weeks after denervation the nerves have lost most of their cholinesterase activity. Some of the enzyme lost from the nerves has collected in sweat ducts (arrows). Acetylthiocholine iodide - CuSO_4 . $\times 130$

nerve fibers were associated with the proximal (coiled) duct. The nerve fibers were connected with the subpapillary nerve plexus of the upper dermis (Fig. 1).

The cholinesterase-positive nerves looked completely normal for the first 3 days after denervation (Fig. 2). Acetylcholinesterase activity started to weaken after the third day after denervation and was gone by the 35th day (Fig. 3).

The eccrine sweat glands themselves remained histologically normal throughout the period of observation. There was a dramatic increase in glycogen (as shown by PAS) in the coiled ducts of the sweat glands in all 3 monkeys after denervation. Two of the monkeys had low glycogen levels in the clear cells of the secretory coils of their palmar sweat glands before denervation. All of the monkeys had high glycogen levels in their clear cells after denervation.

Moderate phosphorylase a activity was seen in the clear cells of the secretory coils both before (Fig. 4) and after (Fig. 5) denervation. Phosphorylase a activity in the coiled ducts varied more within a single skin sample than it did from one sample to another.

Cytochrome oxidase activity was high in the clear cells of the secretory coil and in all cells of the coiled duct both before and after denervation.

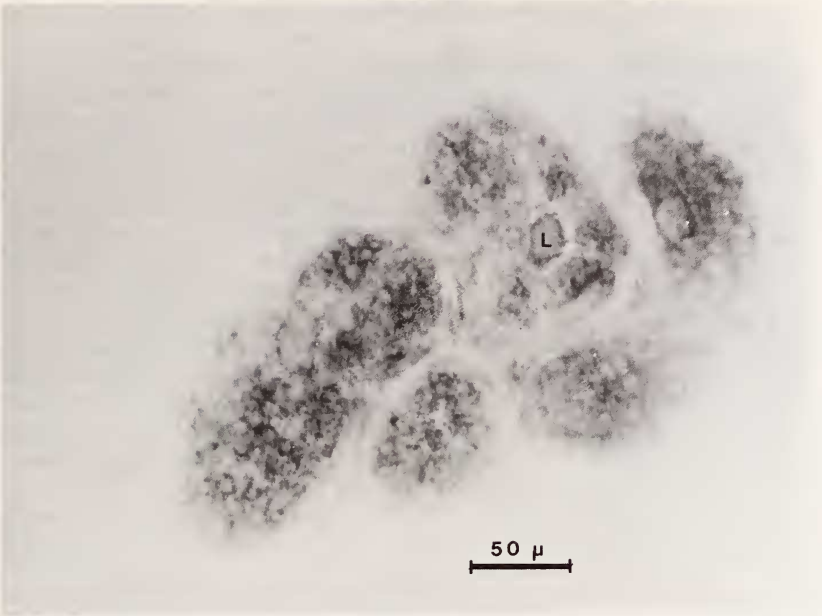


Fig. 4. Phosphorylase a activity in the secretory coil before denervation. Reaction in lumen (L) is a diffusion artefact. Iodine stain. $\times 280$

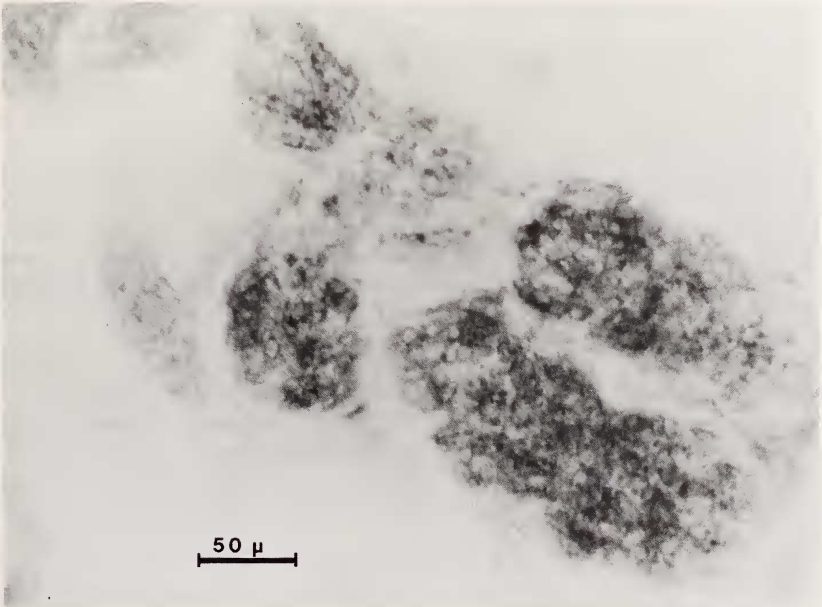


Fig. 5. Phosphorylase a activity in the secretory coil two weeks after denervation. Iodine stain. $\times 280$

Discussion

These results show that the sweat glands of the rhesus monkey slowly lose their capacity to produce visible sweat after denervation. In this, they resemble the sweat glands of man (COON and ROTHMAN 1941) and the African green monkey (*Cercopithecus aethiops*) (SAKURAI and MONTAGNA 1964). The fact that this requires more than a week suggests that the process is one of acclimatization.

The production capacity of the eccrine sweat glands of the patas monkey (*Erythrocebus patas*) increases in hot weather and decreases in cold weather (GISOLFI et al. 1985). The production capacity of human eccrine sweat glands increases after a week of the increased demand of hot weather or hard exercise (DAVIES 1981; TAYLOR 1986) and decreases when cool weather decreases demand (TORII et al. 1991).

It should be expected that the production capacity of sweat glands would decrease in response to the sharply decreased demand following denervation. Acclimatization has been found only in animals that have eccrine sweat glands on the general body surface (COON and ROTHMAN 1941; SAKURAI and MONTAGNA 1964; GISOLFI et al. 1985). Acclimatization is adaptive in a heat dispersal mechanism that may face different challenges over the seasons and over the life cycle.

Animals with eccrine sweat glands only on the palms and soles show denervation hypersensitivity (NAKAMURA and HATANAKA 1958; SAKURAI and MONTAGNA 1965). Denervation hypersensitivity increases sweat production in response to pharmacologic stimuli. Denervation hypersensitivity can be seen only if acclimatization does not occur.

Despite their loss of capacity to produce visible sweat, the denervated sweat glands of the rhesus monkey remained histologically normal. Denervated sweat glands also remain histologically normal in man (SILVER et al. 1964) and the African green monkey (SAKURAI and MONTAGNA 1964). Congenitally aneural eccrine sweat glands are also histologically normal in man (RAFEL et al. 1930; SWANSON 1963).

The denervated sweat glands in this study also continued to show moderate levels of phosphorylase a activity, whose presence is concomitant with sweating (SMITH and DOBSON 1966).

Are denervated sweat glands truly devoid of function? Sympathectomized human patients, who cannot produce visible sweat, produce moist patches where one area of dry skin is held firmly against another (HYNDMAN and WOLKIN 1941). Sweat vaporizing in the secretory coil or in the duct would be invisible and would not stain bromphenol blue paper during the customary one minute exposure, but it would condense on epidermis occluding the duct orifice.

The release of water vapor from the sweat glands would explain why humans with anhydrotic sensory neuropathy, who have aneural sweat glands, suffer less in hot weather than humans with anhidrotic ectodermal dysplasia, who have no sweat glands at all (BOWEN 1957; SWANSON 1963; PINSKY and DiGEORGE 1966). Vapor leaving the duct cools the body just as well as sweat evaporating from the skin surface. Even vapor condensing in the duct transfers heat to the epidermis, increasing radiative and convective cooling from its surface (THIELE et al. 1972; REAY and THIELE 1977).

Vapor sweating allows an animal with thick fur to cool itself efficiently. Thus, the possession of sweat glands on the general body surface is adaptive for a mammal that is diurnally active in hot weather. Thermoregulatory eccrine sweat glands have evolved in Scandentia (MONTAGNA et al. 1962) and among the Primates, both of which are diurnal and primarily tropical.

The absence of eccrine sweat glands from the general body surface of the diurnal lemurs (SOKOLOV 1982) and marmosets (PERKINS 1966; PERKINS 1968) suggests that the presence of eccrine sweat glands on the general body surface is not a primitive feature of primates. Although tree shrews have eccrine sweat glands on the general body surface (MONTAGNA et

al. 1962), it is now generally agreed that the tree shrews are not primates (VAN VALEN 1965; DENE et al. 1966; MARTIN 1982).

The higher New World monkeys are diurnal and have eccrine sweat glands on the general body surface (MACHIDA et al. 1967; PERKINS and MACHIDA 1967). Since marmosets and the other monkeys of the New World have a common ancestor that is not shared with the Old World monkeys, the extension of eccrine sweat glands to the general body surface must have evolved after the divergence of Old and New World primates (PERKINS 1968; MACHIDA et al. 1967). The absence of eccrine sweat glands from the skin of the tarsier which shares a common ancestor with the Old World monkeys tends to confirm this (MONTAGNA and MACHIDA 1966).

The presence of eccrine sweat glands on the general body surface of Scandentia, Cebidae, and Old World monkeys and apes is a result of parallel evolution (MONTAGNA and PARAKKAL 1974; FOLK and SEMKEN 1991). The repeated evolution of thermoregulatory eccrine sweat glands is due to the survival value of vapor sweating.

Denervated sweat glands probably produce water vapor under the influence of circulating epinephrine. Although atropine prevents the appearance of liquid sweat detectable by the starch-iodine test or bromphenol blue paper, it does not prevent the delivery of water vapor to a sudorimeter (WOLF and MAIBACH 1974). Although some of the sudorimotor nerves secrete norepinephrine (UNO and MONTAGNA 1975), the eccrine sweat glands have more β receptors than α receptors and are more sensitive to epinephrine (SATO and SATO 1981). Physiological levels of circulating epinephrine increase evaporative heat loss in the intact stump-tail macaque, *Macaca speciosa* (ROBERTSHAW et al. 1973). Higher levels of epinephrine can elicit visible sweat in man (WADA 1950; CHALMERS and KEELE 1951).

In 1935 OGATA found that some human sweat glands failed to produce liquid sweat under any stimulus (KUNO 1956). This suggests that many innervated sweat glands produce only vapor. The excess of sweat glands in histological counts in man (MONTAGNA and PARAKKAL 1974) and rhesus monkeys (SMITH 1969) over counts of sweat droplets on the skin of man (MONTAGNA and PARAKKAL 1974) and rhesus monkeys (JOHNSON and ELIZONDO 1974) leads to the same conclusion.

Denervated sweat glands remain histologically normal because they continue to produce water vapor, which is part of the normal function of sweat glands.

Acknowledgements

The experimental part of this work was done in 1969 at the Oregon Regional Primate Research Center with support from grants FR 00163 and AM 05512 from the National Institutes of Health and grant 5T01GM00445-09 from the US Public Health Service.

The author thanks Dr. WILLIAM MONTAGNA for his advice and encouragement, Dr. CLARENCE DENNOY for his expert surgery, and JOHN OCHSNER of the Oregon Regional Primate Research Center for his special efforts in the care of the research animals. He also thanks PIA YORK of Barry University for her editorial assistance.

Zusammenfassung

Auswirkungen von Denervierung der Schweißdrüsen bei Rhesusaffen, Macaca mulatta

Die Schweißdrüsen des Rhesusaffen sind umgeben von cholinergen Nerven, die vom subpapillaren Plexus stammen. Nach Durchtrennung der Nerven von Schweißdrüsen bildet sich kein Schweiß mehr. Während der nächsten zwei Wochen verliert die Schweißdrüse ihre Fähigkeit auf Arzneien zu reagieren. Im Laufe eines Monats werden die Nerven degeneriert. Nach Durchtrennung der Nerven von menschlichen Schweißdrüsen ergeben sich ähnliche Effekte.

Die denervierte Schweißdrüse bleibt histologisch jedoch unverändert. Zusätzlich ist auch eine Aktivität der Phosphorylase zu beobachten, die normal mit der Schweißproduktion verbunden ist. Das deutet an, daß die denervierten Schweißdrüsen Wasserdunst produzieren. Andere Studien an Schweißdrüsen von Affen und Menschen beweisen, daß denervierte und normale Schweißdrüsen Wasserdunst absondern.

Die Schweißabsonderung bei Säugetieren mit Fell hat einen kühlenden Effekt. Das Ausbreiten der

Schweißdrüsen von Handfläche und Sohle zur Oberfläche des Körpers begünstigte sehr wahrscheinlich die evolutive Radiation der höheren Affen.

Literature

- BOWEN, R. (1957): Hereditary ectodermal dysplasia of the anhidrotic type. *South. Med. J.* **50**, 1018-1021.
- BURSTONE, M. S. (1961): Modifications of histochemical techniques for demonstration of cytochrome oxidase. *J. Histochem. Cytochem.* **9**, 59-65.
- CHALMERS, T. M.; KEELE, C. A. (1951): Physiological significance of the sweat response to adrenaline in man. *J. Physiol. (Lond.)* **114**, 510-514.
- COON, J. M.; ROTHMAN, S. (1941): Sweat response to drugs with nicotine-like action. *J. Pharmacol. Exper. Ther.* **73**, 1-11.
- DALE, H. H.; FELDBERG, W. (1934): The chemical transmission of secretory impulses to the sweat glands of the cat. *J. Physiol. (Lond)* **82**, 121-128.
- DAVIES, C. T. M. (1981): Effect of acclimatization to heat on the regulation of sweating during moderate and severe exercise. *J. Appl. Physiol.* **50**, 741-746.
- DENE, H. T.; GOODMAN, M.; PRYCHODKO, W. (1976): Immunodiffusion evidence on the phylogeny of the primates. In: *Molecular anthropology*. Ed. by M. GOODMAN, R. E. TASHJIAN, and J. H. TASHJIAN. New York: Plenum. Pp. 171-195.
- ERÄNKÖ, O.; PALKAMA, A. (1961): Improved localization of phosphorylase by the use of polyvinylpyrrolidone and high substrate concentration. *J. Histochem. Cytochem.* **9**, 585.
- FOLK, G. E.; SEMKEN, H. A., Jr. (1991): The evolution of sweat glands. *Int. J. Biometeorol.* **35**, 180-186.
- GISOLFI, C. V.; SATO, K.; WALL, P. T. (1985): Monkey model and techniques for studying eccrine sweating in man. In: *Models in dermatology*. Ed. by H. I. MAIBACH and N. J. LOW. New York, Basel: Karger. Pp. 117-125.
- GOMORI, G. (1952): *Microscopic histochemistry*. Chicago: University of Chicago Press.
- HYNDMAN, O. R.; WOLKIN, J. (1941): Sweat mechanism in man: study of distribution of sweat fibers from sympathetic ganglia, spinal roots, spinal cord and common carotid artery. *Arch. Neurol. Psychiat.* **45**, 446-467.
- JOHNSON, G. S.; ELIZONDO, R. S. (1974): Eccrine sweat gland in *Macaca mulatta*: physiology, histochemistry, and distribution. *J. Appl. Physiol.* **37**, 814-820.
- KUNO, Y. (1956): *Human perspiration*. Springfield, IL: Thomas.
- LILLIE, R. D. (1954): *Histopathologic technic and practical histochemistry*, 2nd ed. New York: McGraw-Hill.
- MACHIDA, H.; GIACOMETTI, L. (1968): The skin. In: *Biology of the howler monkey*. Ed. by M. R. MALINOW. Basel: Karger. Pp. 126-140.
- MACHIDA, H.; PERKINS, E.; HU, F. (1967): The skin of primates. XXXV. The skin of the squirrel monkey (*Saimiri sciurea*). *Am. J. Phys. Anthrop.* **26**, 45-54.
- MACHIDA, H.; PERKINS, E.; MONTAGNA, W. (1964): The skin of primates. XXIII. A comparative study of the skin of the green monkeys (*Cercopithecus aethiops*) and the Sykes monkey (*Cercopithecus mitis*). *Am. J. Phys. Anthrop.* **22**, 453-465.
- MARTIN, R. D. (1982): Et tu, tree shrew? *Natural History* **91** (8), 26-33.
- MONTAGNA, W.; MACHIDA, H. (1966): The skin of primates. XXXII. The Philippine Tarsier (*Tarsius syrichta*). *Am. J. Phys. Anthrop.* **25**, 71-84.
- MONTAGNA, W.; PARAKKAL, P. (1974): *The structure and function of skin*. 3rd ed. New York: Academic Press.
- MONTAGNA, W.; YUN, J. S. (1964): The skin of primates. VIII. The skin of the Anubis baboon (*Papio doguera*). *Am. J. Phys. Anthrop.* **20**, 131-141.
- MONTAGNA, W.; YUN, J. S.; MACHIDA, H. (1964): The skin of primates. XVIII. The skin of the rhesus monkey. *Am. J. Phys. Anthrop.* **22**, 307-320.
- MONTAGNA, W.; YUN, J. S.; SILVER, A. F.; QUEVEDO, W. C., Jr. (1962): The skin of primates. XIII. The skin of the tree shrew (*Tupaia glis*). *Am. J. Phys. Anthrop.* **20**, 431-440.
- NAKAMURA, Y.; HATANAKA, K. (1958): Effect of denervation of the cat's sweat glands on their responsiveness to adrenaline, nicotine, and mecholyl. *Tohoku. J. Exper. Med.* **68**, 225-237.
- PINSKY, L.; DIGEORGE, A. M. (1966): Congenital familial sensory neuropathy with anhidrosis. *J. Pediat.* **68**, 1-13.
- PERKINS, E. M., Jr. (1966): The skin of primates. XXXI. The skin of the black-collared tamarin (*Tamarinus nigricollis*). *Am. J. Phys. Anthrop.* **25**, 41-70.
- (1968): A histological and histochemical survey of the skin of New World monkeys. Ph. D. thesis, Univ. Oregon Medical School, Portland.
- PERKINS, E. M.; MACHIDA, H. (1967): The skin of primates. XXIV. The skin of the golden spider monkey (*Ateles geoffroyi*). *Am. J. Phys. Anthrop.* **26**, 35-43.

- RAFEL, E.; ALBERCA, R.; BAUTISTA, J.; NAVARRETE, M.; LAZO, J. (1930): Congenital insensitivity to pain with anhidrosis. *Muscle and Nerve* 3, 216–220.
- REAY, D. A.; THIELE, F. A. J. (1977): Heat pipe theory applied to a biological system: quantification of the role of the "resting" eccrine sweat gland in thermoregulation. *J. Theor. Biol.* 64, 789–803.
- ROBERTSHAW, D.; TAYLOR, C. R.; MAZZIA, L. M. (1973): Sweating in primates: secretion by the adrenal medulla during exercise. *Am. J. Physiol.* 224, 678–681.
- SAKURAI, M.; MONTAGNA, W. (1964): Pharmacological properties of the sweat glands of *Lemur mongoz*. *J. Invest. Dermatol.* 42, 411–414.
- — (1964): Experiments in sweating on the palms of the green monkey (*Cercopithecus aethiops*). *J. Invest. Dermatol.* 43, 279–285.
- — (1965): Observations on the sweat glands of *Lemur mongoz* after denervation. *J. Invest. Dermatol.* 44, 87–92.
- SATO, K.; SATO, F. (1981): Pharmacologic responsiveness of isolated single eccrine sweat glands. *Am. J. Physiol.* 240, R44–51.
- SILVER, A.; MONTAGNA, W.; VERSACI, A. (1964): The effect of denervation on sweat glands and Meissner corpuscles of human hands. *J. Invest. Dermatol.* 42, 307–324.
- SILVER, A.; VERSACI, A.; MONTAGNA, W. (1963): Studies of sweating and sensory function in cases of peripheral nerve injuries of the hand. *J. Invest. Dermatol.* 40, 243–258.
- SMITH, A. A. (1969): The palmar sweat glands of the rhesus monkey. Ph. D. thesis, University of Oregon Medical School, Portland.
- SMITH, A. A.; DOBSON, R. L. (1966): Sweating and glycogenolysis in the palmar eccrine sweat glands of the rhesus monkey. *J. Invest. Dermatol.* 47, 313–316.
- SMITH, A. A.; PERKINS, E. M.; MACHIDA, H. (1966): Durable mounts of the iodine stain for the phosphorylase reaction. *Stain. Technol.* 41, 346–348.
- SWANSON, A. G. (1963): Congenital insensitivity to pain with anhidrosis. *Arch. Neurol.* 8, 299–306.
- TAYLOR, N. A. (1986): Eccrine sweat glands. Adaptations to physical training and heat acclimation. *Sports Med.* 3, 387–397.
- THIELE, F. A. J.; HEMELS, H. G. M.; MALTEN, K. E. (1972): Skin temperature and water loss by skin. *Trans. St. John's Hospital Dermatol. Soc.* 58, 218–223.
- TORII, M.; YAMASAKI, M.; SASAKI, T.; NAKAYAMA, H. (1991): Functional differentiation of thermoregulation. *Ann. Physiol. Anthropol.* 10, 163–170.
- UNO, H.; MONTAGNA, W. (1975): Catecholamine-containing nerve terminals of the eccrine sweat glands of macaques. *Cell. Tiss. Res.* 58, 1–13.
- VALEN, L. VAN (1965): Treeshrews, primates, and fossils. *Evolution* 19, 137–151.
- WADA, M. (1950): Sudorific action of adrenaline on the human sweat glands and determination of their excitability. *Science* 111, 376–377.
- WOLF, J. E.; MAIBACH, H. I. (1974): Palmar eccrine sweating – the role of adrenergic and cholinergic mediators. *Brit. J. Dermatol.* 91, 439–446.

Author's address: Dr. ALLEN A. SMITH, School of Podiatric Medicine, Barry University, Miami Shores, FL 33161, USA